Decreased Cerebrovascular Response to CO₂ in Post-menopausal Women with COPD: Role of Oxidative Stress

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Chronic obstructive pulmonary disease (COPD) is associated with cerebrovascular abnormalities and an overproduction of reactive oxygen species. We hypothesized that COPD patients have oxidant-related cerebrovascular dysfunction. The main objective was to evaluate cerebrovascular reactivity and its relationship to oxidative stress in women with COPD.

We studied eight women with moderate-COPD, and ten healthy women control subjects of similar age. Transcranial Doppler ultrasound assessed cerebral blood flow velocity during hypercapnia. Plasma was assessed at rest for DNA oxidation, advanced oxidation protein products, lipid peroxidation, nitrotyrosine, antioxidant enzyme activity (glutathione peroxidase and catalase), and end-product of nitric oxide metabolism.

Moderate-COPD patients showed decreased cerebrovascular sensitivity to CO₂ (COPD: 1.17 ± 0.54 versus Control: 2.15 ± 0.73 cm·sec⁻¹·mmHg⁻¹; P < 0.01). COPD patients to have higher levels of DNA and lipid oxidation, advanced oxidation protein products, and higher glutathione peroxidase activity (P<0.05). Controlling for measures of oxidative stress (DNA and lipid oxidation, advanced oxidation protein product) eliminates statistically significant differences between the COPD and control groups are eliminated in the cerebral blood flow sensitivity to CO₂.

Women with moderate COPD have cerebrovascular dysfunction. Our results suggest that increased levels of systemic oxidative stress may have implications in the cerebrovascular dysfunction observed during hypercapnia in COPD.

Abstract word count: 200
INTRODUCTION

Most recently, COPD is gaining attention for the consequential systemic manifestations and co-morbidities resulting from the disease [1]. Although these patients are at a 3-4 fold increase in the risk of developing cardio- and cerebro-vascular disease, limited information is available regarding the pathophysiology relating to vascular diseases in COPD. Extrapulmonary consequences, including altered arterial blood gas levels, pH imbalance, increased oxidative stress, vascular dysfunction and autonomic disturbances, all have potential to alter the regulation of cerebral blood flow (CBF). In healthy individuals, CBF and ventilation increase linearly with arterial $P_{CO_2}$ ($PaCO_2$). It is well established that COPD patients have a decreased ventilatory output to CO$_2$ [2-4]. However, few studies have investigated the cerebrovascular response to CO$_2$ in these subjects, and how decreased ventilation can affect this response. Recent literature suggests that cerebrovascular sensitivity and ventilatory response are tightly linked in healthy individuals [5]. However, evidence suggests that COPD patients exhibit cerebrovascular disturbances [3, 6, 7], although the pathologic onset and cause of these disturbances have not yet been comprehensively studied.

Furthermore, the role of oxidative stress pertaining to cerebrovascular dysfunction is of particular interest in COPD patients. COPD is associated with an overproduction of reactive oxygen and nitrogen species (ROS and RNS, respectively), leading to an imbalance of oxidants-antioxidants, resulting in oxidative stress [8]. ROS have been implicated in the role of vascular dysfunction both directly (via dilatory effects of H$_2$O$_2$), and indirectly through decreased bioavailability of nitric oxide (NO) via the promotion of superoxide anion quenching to form
peroxynitrite [9]. By the reduction of available NO, this reaction (i.e. $O_2^- + NO \rightarrow ONOO^-$) can subsequently affect cerebrovascular tone, resulting in vasoconstriction [10].

While a few studies have looked at cerebrovascular health in COPD patients [3, 6, 7, 11], no study has investigated how oxidative stress and antioxidant activity could be involved in the CBF regulation observed in COPD. Estrogen is a sex-hormone with beneficial vasoactive and antioxidant properties. After menopause estrogen sharply declines, and oxidative stress is reported to be increased [12]. This may have an impact on women with COPD who are already at increased risk of exposure to oxidants.

Furthermore, the present collection of literature relating to cerebrovascular physiology and COPD represents exclusively male, or mixed gender studies. Recent commentary states the impact of COPD in women is significantly understudied, and evidence that does exist, suggests important gender differences, raising debate as to the role of gender as a potential risk factor for developing COPD[13]. Evidence suggests that women are at increased susceptibility for the development of the disease (e.g., incur COPD after fewer number of cigarettes/lifetime compared to men), have increased prevalence rates, and exhibit the poorest outcomes associated with COPD [14].

We therefore choose to exclusively study women to gain better understanding of certain physiological effects of COPD in this under-represented group. Thus, we sought to determine: 1) the extent to which cerebrovascular regulation is altered in women with moderate-COPD, and whether there is a relationship to ventilation, and 2) whether the expected differences in cerebral blood flow are explained by systemic oxidative stress markers, or antioxidant activity. Some of these results have been previously presented in an abstract form [15].
METHODS

Study Participants

Ten post-menopausal women with smoking-related COPD, and twelve healthy, non-smoking post-menopausal women (controls) were recruited for participation in this study. COPD subjects were recruited from participating outpatient medical clinics within the Calgary Health Region, and controls were recruited from the community. All study participants visited the Laboratory of Human Cerebrovascular Physiology at the University of Calgary, Alberta (1103 m elevation above sea level) for two testing sessions. Subjects were instructed to refrain from eating or drinking 4 hours prior to each testing session. The study was approved by the institutional Conjoint Health Research Ethics Board and conformed to the Declaration of Helsinki, and all participants provided written, informed consent.

Major Inclusion Criteria. Patients had physician-diagnosed COPD, with a smoking history >10 pack-years and airflow obstruction (FEV₁/FVC <70%; FEV₁ ≤ 70% predicted). Patients were all ex-smokers (>1 year), post-menopausal for ≥ 12 months, able to walk independently outside or on stairs, and had a BMI < 35 kg·m⁻². Participating controls were healthy volunteers, with no history of lung disease, or regular cigarette smoking (< 1 pack-year). Complete exclusion criteria are listed in the online supplement.

Experimental Protocol

Participants visited the laboratory on two occasions. The first day consisted of a medical screening questionnaire, pulmonary function testing, and venous blood collection. After approximately 1 week, subjects returned for a CO₂-challenge test, which was conducted in most participants between 11:00 am - 2:00pm.
Pulmonary Function Test. Spirometry, measures of lung volumes and single-breath diffusion capacity were completed in all subjects, as per ATS guidelines [16-18].

Protocol to measure the cerebrovascular and ventilatory response to euoxic hypercapnia. Subjects were comfortably seated in a semi-recumbent position at rest for 10 minutes for collection of baseline vascular and ventilatory variables. An arterialized capillary blood sample was taken from the middle finger after warming for 3-minutes. Blood samples were immediately analyzed for PO2, PCO2, and acid-base balance (Radiometer ABL 725, Denmark). Briefly, using dedicated software (BreatheM v2.38, University Laboratory of Physiology, Oxford, UK), the technique of dynamic end-tidal forcing [19], was used to precisely target the desired end-tidal pressure CO2 (PETCO2) and end-tidal pressure of oxygen (PETO2). The ~10-minute hypercapnic protocol progressed to hypercapnia at +9 mmHg above the resting PETCO2, while PETO2 was held constant at baseline values. The protocol was designed as 5 x 2-minute stages of increasing PETCO2, in a stepwise fashion (i.e., +1, +3, +5, +7, +9 mmHg above eucapnia).

Heart rate, oxygen saturation, and continuous beat-beat blood pressure via finger pulse photoplethysmography was measured. Respiratory volumes were measured with a turbine and volume transducer (VMM-400; Interface Associates, Laguna Niguel, CA), and respiratory flow direction and timing were obtained with a pneumotachograph (RSS100-HR, Hans Rudolf, Kansas City, MO, USA). Middle cerebral artery (MCA) blood flow velocity was continuously measured using a 2-Hz pulsed Doppler Ultrasound system (TC22, SciMed, Bristol, England). Peak blood flow velocity (Vp) was used as a surrogate for global cerebral blood flow (CBF). The power (P) signal acquired from the Doppler system is used as an indicator for changes in vessel diameter. Therefore, without a change in P, Vp is considered to be a reliable index of flow.
**Biochemical Analyses.** Venous blood was collected at rest into two EDTA, and two SST tubes for biochemical analysis. Blood was centrifuged at 3200 rpm for 10 minutes at 4°C. Plasma and serum were separated into appropriate aliquots and frozen at -80°C until assays were performed. Assays to measure plasma levels of oxidative stress (8-hydroxy-2′-deoxyguanosine [8-OHdG], malondiadehide [MDA], advanced oxidation proteins product [AOPP]) nitrosative stress (nitrotyrosine), antioxidant enzyme activity (glutathione peroxidase [GPX] and catalase activities), and end-product of nitric oxide (nitrites and nitrates) were performed. Hormone and cholesterol analysis was performed by Calgary Laboratory Services. Further procedural details are provided in the Online Supplement.

**Data and Statistical Analysis**

The main outcome variable is the cerebrovascular sensitivity to CO₂. Secondary outcomes include the ventilatory and the blood pressure response to CO₂, and molecular markers related to vascular function (oxidative stress, antioxidants, and NOx). An estimate of the cerebrovascular sensitivity (i.e., $\bar{V}P$ sensitivity) to CO₂ was calculated for each individual as the slope of the regression relating $\bar{V}P$ versus $PETCO₂$, during hypercapnia. Likewise, the ventilatory sensitivity (i.e., $\bar{V}E$ Sensitivity) to CO₂ was calculated as the regression slope of $\bar{V}E$ against $PETCO₂$ during hypercapnia. The last 30-seconds of data of each hypercapnic stage was averaged and used in this calculation. Where percent changes are reported, this data is normalized to the last 3-minutes of the isocapnia euoxia baseline period preceding the hypercapnic steps.

Using the variability (SD = 0.57 cm·sec⁻¹·mmHg⁻¹) and expected between-group difference of 0.75 cm·sec⁻¹·mmHg⁻¹ from a previously published study [7], we determined that to detect a significant difference in the cerebrovascular sensitivity to hypercapnia, each group would consist of 11 participants (using a two-tailed test and setting $\alpha = 0.05$ and $\beta = 0.80$).
Tests of normality and homogeneity of variance were performed and confirmed the appropriate use of parametric statistical procedures (SPSS Version 17.0, SPSS Inc., Chicago, IL). The CO₂-sensitivity indices for CBF and \(V_E\) were compared between groups using an independent t-test. The main effect of “hypercapnia” and the “group x hypercapnia” interaction was evaluated on physiological variables (respiratory \([\text{PETCO}_2, \text{PETO}_2, V_E, Bf, VT, V_E/MVV}\], cardiovascular \([\text{MAP, HR, SaO}_2]\], and cerebrovascular \([\bar{V}_p, \text{CVC}]\)) using a repeated measure analysis of variance. The repeated effect of each CO₂ stage (i.e., +1, +3, +5, +7, +9) was evaluated on the aforementioned physiological variables. Individual cerebrovascular and ventilatory sensitivities were ranked, and Spearman’s correlation coefficient was used to evaluate the strength of the relationship between \(V_E\) and \(\bar{V}_p\) sensitivity. Group differences in molecular markers (i.e., oxidants, antioxidants, and NOx metabolism) were assessed using independent t-tests. Post-hoc analysis assessed relationships between these molecular markers and main cardio- and cerebro-vascular outcome variables using Pearson’s correlations. Using exploratory analyses, we used several baseline factors (blood pressure, age, oxidative stress, antioxidant enzyme status, NO-metabolism) as covariates in a one-way ANCOVA to find the best “adjusted” model to explain the variance in \(\bar{V}_p\) sensitivity to CO₂ between groups. Data are presented as mean ± SD, and significance was set at \(\alpha\)-level \(\leq 0.05\). Confidence intervals are calculated at 95%.
RESULTS

Subject Characteristics

Data were not obtained in 2 subjects (1 control and 1 COPD) due to lack of a suitable MCA signal. Furthermore, data from 1 COPD patient was excluded because the patient did not complete the CO₂ challenge to entirety, due to overwhelming breathlessness. A blood sample was not obtained in 1 control. Therefore, we analyzed data from 10 controls, and 8 COPD patients.

Physical characteristics and pulmonary function tests of participants are summarized in Table 1. Measurements of resting blood gases acquired by end-tidal, and via capillary blood is summarized in Table 2.

Physiological Responses to Hypercapnia

At rest, there were no significant differences between main vascular outcomes (i.e., \( \bar{V}_P \), MAP, heart rate) between COPD patients and controls (Table 3). In response to hypercapnia, the vascular response in COPD patients was blunted, as evident by only a 19% increase in \( \bar{V}_P \) (vs 37% in controls; \( P < 0.01 \)), and an 8% increase in MAP (vs 12% in controls; \( P > 0.05 \)) from baseline (Fig 1A and 1B). The mean \( \bar{V}_P \) sensitivity (unadjusted) for COPD patients is significantly decreased (COPD: 1.17 ± 0.54 versus Control: 2.15 ± 0.73 cm·sec⁻¹·mmHg⁻¹; \( P < 0.01 \)) (Fig 2A).

Ventilatory output was decreased in COPD patients at the end of the CO₂ challenge (\( P \leq 0.05 \)) (Figure 1C), however, there was only a trend towards a statistical difference when considering the \( V_E \) sensitivity to CO₂ (0.90 ± 0.61 versus 1.83 ± 1.26 L·min⁻¹·mmHg⁻¹; \( P = 0.07 \)) (Fig 2B). Increases in ventilation were achieved primarily by an increase in tidal volume (\( V_{TE} \)), rather than breathing frequency (\( B_{f} \)) (Table 3). Using Inspiratory flow (\( V_{TI}/T_{I} \)) as an indication
of respiratory drive, COPD patients showed a similar drive to breathe at rest between compared to controls, whereas \( V_{T_i}/T_i \) was reduced during hypercapnia in COPD patients (Table 3).

Each individual \( \bar{V}_p \) and \( \bar{V}_E \) sensitivity score was ranked amongst all participants. There was a significant positive correlation between the ranking of ventilatory and cerebrovascular sensitivity to hypercapnia. Subjects with low cerebrovascular sensitivity to CO\(_2\) had a corresponding low ventilatory sensitivity, and vice versa (\( r = 0.61; \ P < 0.01 \)).

**Biochemical Analysis: Oxidative Stress, Antioxidant Enzyme Activity, and NO Level**

**Oxidative Stress and Antioxidant Activity**

Results of biochemical assays are summarized in Table 4. COPD patients showed significantly higher levels of oxidative stress as indicated by increased plasma 8-OHdG, MDA, and AOPP (\( P \leq 0.05 \)). These patients also had significantly higher antioxidant enzyme activity in the form of GPX (\( P \leq 0.01 \)). The ratio between oxidative stress and antioxidant activity (i.e., 8-OHdG and GPX) was significantly higher in COPD patients than controls (1.03 ± 0.50 *versus* 0.62 ± 0.23, respectively) (\( P \leq 0.05 \)).

**Vascular Parameters and Oxidative Stress**

We performed (with-in group) correlation analysis between plasma oxidative stress/antioxidant markers, and vascular outcomes to measure the strength of the relationship between these variables. We did not find any significant relationships between oxidative stress (8-OHdG, AOPP, MDA, and nitrotyrosine) and \( \bar{V}_p \) sensitivity, MAP, or cerebrovascular conductance. However, COPD patients with higher catalase activity were found to be associated with higher \( \bar{V}_p \) sensitivity (\( r^2 = 0.59; \ P < 0.05 \)).

A one-way ANCOVA was conducted using “group” (COPD or control) as the fixed factor, and cerebrovascular sensitivity as the dependent variable. A preliminary analysis evaluating the homogeneity of regression (slopes) assumption indicated the relationship between
the co-variates and dependent variable did not differ significantly ([8-OHdG: F(1,14) = 1.45, p = 0.248]; AOPP: F(1,14) = 0.466, p = 0.506]; [MDA: F(1,14) = 0.081, p = 0.780]). Using these co-variates, the ANCOVA was non-significant, F(1,13) = 0.033, p = 0.858, thus eliminating the difference in cerebrovascular sensitivity previously observed between groups (COPD: 1.65 ± 1.08 cm·sec⁻¹·mmHg⁻¹ versus controls: 1.76 ± 1.01 cm·sec⁻¹·mmHg⁻¹) (Fig 2A).

Based on our findings that suggest a potential role between OS and cerebrovascular dysfunction in women with COPD, we incorporated additional statistical analyses to offer a clinical perspective. Using the adjusted model, participants were assigned to either “COPD”, or “control” group, according to the plasma levels of oxidative stress. Positive predictive, and negative predictive values (PPV and NPV, respectively) were calculated, where the prevalence rate of COPD was 44% (8/18). Individuals were identified to have “COPD” if the level of plasma oxidative stress was above the expected mean concentration (of either 8-OHdG, MDA, or AOPP). Individuals were identified as “healthy/control” if all three levels of OS markers were below the adjusted mean OS concentration. Results indicate the PPV to be 93.5%, and the NPV to be 100%.
DISCUSSION

We present novel data indicating a link between cerebrovascular reactivity, and measures of systemic oxidative stress in COPD patients. The major finding in this study is an impaired cerebrovascular response to hypercapnia in women with COPD. We report a blunted cerebrovascular dilatory response in most, but not all COPD patients, even at modest levels of CO₂ administration. As predicted, higher levels of systemic oxidative stress markers were found in the COPD patient cohort. As suggested in other populations [10, 20], in this context increased oxidative stress could explain the differences observed between the cerebrovascular sensitivity to hypercapnia observed between COPD patients, and healthy control subjects.

Physiologic response to hypercapnia

It is well known that in a healthy population, increased PaCO₂ induces cerebrovascular dilation, leading to an increase in cerebral blood flow [21]. This response depends on several co-operative pathways: 1) the chemical stimulus (pH/H⁺) at the central chemoreceptors, 2) the ventilatory response (e.g., respiratory muscles), and 3) the vasodilatory response in the small vessels of the cerebral circulation. Insufficiencies at any one of these levels, can lead to an abnormal hypercapnic response.

Our findings show COPD patients to have a lower CBF response to CO₂ compared to healthy controls (+19% versus +41%, respectively). We did not find evidence for chronic cerebrovascular dilation in COPD patients. Our results are supported in both an animal model [22], as well as in hypercapnic patients, with severe COPD [6]. Cigarette smoking is known to induce both acute and chronic cerebrovascular dilation in healthy individuals, but cerebrovascular reactivity appears to be maintained. In young adults, cerebrovascular deficiencies are only observed following acute smoking (1-minute) [23]. Similarly, in a healthy older population, smoking status was not a significant factor in determining the sensitivity to
hypercapnia [7]. Interestingly, in this same study, Bernardi et al. found that individuals who were current smokers and had mild COPD, had significantly lower cerebrovascular sensitivity to hypercapnia compared to individuals who only had mild airflow obstruction, without a smoking history. Overall, however, the cerebrovascular reactivity in mild COPD patients did not differ from matched healthy controls. We now show that normocapnic patients with moderate COPD show cerebrovascular abnormalities. We believe that frequent stimuli specific to individuals with COPD (e.g. cigarette smoking, occurrence of frequent arterial oxygen desaturations) may exhaust the normal vascular response via constant vaso-dilation/constricting cycles. Acute effects of smoking cause an immediate constriction of the pial arteries, followed by vasodilation which is likely mediated by nicotine that stimulates NO release [24]. Extensive reviews on the topic suggest that increased oxidative stress may lead to either a decreased generation, or bioavailability of NO leading to vasomotor dysfunction, specific to the vascular endothelium [25, 26].

We found that patients with COPD showed a trend towards decreased ventilatory response to hypercapnia. Both mechanical (respiratory) limitations and desensitization of the central chemoreceptor have been implicated in the explanation of this pathology. A decreased ventilatory response provides an avenue for increased cerebral dilation in COPD participants. This is contrary to what we observed in patients, as the cerebrovascular response was blunted. In healthy individuals, Xie and colleagues [27] showed that decreased cerebrovascular responsiveness to CO₂ stimulates the ventilatory response, suggesting that cerebrovascular sensitivity to CO₂ has great influence on the V̇E responsiveness of the central chemoreceptors. It is possible that the mechanisms involved in the control of breathing and cerebrovascular regulation in COPD patients are independently altered (i.e., two separate mechanisms affecting these outcomes).
Molecular markers

Oxidative Stress and Antioxidant Status

Oxidative stress represents an unfavorable imbalance between reactive oxygen species and antioxidants, either from the overproduction of oxidants, or the depletion of antioxidants. In addition to endogenous sources of ROS (normal cellular metabolism), COPD patients are exposed to exogenous forms of free radicals from environmental pollutants and/or cigarette smoke. Our findings indicate a significant increase in both systemic oxidative stress markers (8-OHdG, MDA, and AOPP), and increased antioxidant enzyme activity (GPX) in COPD patients, compared to healthy control subjects, and are in similar agreement with other published data [28-31]. In contrast to our results, increased glutathione has previously been shown to be negatively correlated with lung function in patients with chronic airflow limitation [30]. We suspect that there may be an adaptive response involved in the oxidative stress-antioxidant enzyme response, and that the high level of oxidants stimulates the antioxidant enzymatic system in an effort to counteract the high burden of oxidants. Recent reports suggest a decrease in antioxidant capacity in both healthy smokers and patients with COPD, when compared to non-smoking controls [29, 32]. Furthermore, antioxidant status was not different between current or ex-smokers in either the healthy or those with COPD in these groups, implying that the disease state itself is a determinant of systemic oxidative stress, rather than current smoking habit [32].

Nitric Oxide and Vascular Parameters

Smoking is known to alter NO bioavailability [33] and cause endothelial dysfunction [34], however, less is known how this affects COPD patients. NO is produced by the conversion of L-Arginine to L-citrulline in the presence of NO synthases. One possible explanation for the decrease in NOx that we observed is that increased ROS (superoxide anion, O2−) reacts with NO, forming peroxynitrite (ONOO−), consequently leading to the formation of 3-nitrotyrosine,
thereby reducing the availability of NO. Although nitrotyrosine tended to be higher in COPD (P = 0.11), we did not find a significant negative correlation between NOx and nitrotyrosine, as expected, suggesting that other mechanisms regulate NO metabolism such as NO-synthase [35].

We have previously shown in healthy older women that higher levels of NOx are associated with a decrease in resting arterial blood pressure [20], however, resting MAP did not differ between COPD patients and control subjects, and thus no relationship was found between NOx and MAP. This same study [20] suggests that in a healthy aging population, increased ROS and peroxynitrite may in part be a detrimental contributor in the determination of cerebrovascular tone. We anticipated NOx to have a greater involvement in the cerebrovascular indices measured, since it is known that tone of cerebral blood vessels is influenced by NO under resting conditions, and the loss of NO bioavailability produces vasoconstriction [10]. Furthermore, increases in CBF during hypercapnia also appear to be dependent on production on NO [36].

**Limitations**

A limitation of our study is the use of end-tidal measurements used as an indication of arterial gas concentrations. Caution should be taken in making this comparison, particularly in elderly populations and individuals with chronic lung disease due to widened alveolar-arterial gradients. To account for this limitation, we obtained capillary blood samples to measure blood gases at rest. When considering PCO₂, H⁺, and HCO₃⁻, good agreement has been shown between arterial and capillary samples in patients with chronic lung disease [37]. In COPD patients, we found that end-tidal PCO₂ was significantly lower than capillary PCO₂ (PcCO₂), consistent with increased alveolar deadspace, and a widened alveolar-arterial gradient. However, based on the PcCO₂, we can conclude that on average, the COPD group is not chronically hypercapnic.

Direct arterial blood samples are invasive and at the risk of compromising patient recruitment, were not included in our study.
Our sample size was calculated to detect differences in our main outcome variable (i.e., \( \bar{V}_P \) sensitivity to CO\(_2\)). We do however acknowledge that our sample size may be insufficient to detect correlational differences between oxidative stress markers and measures of cerebrovascular function, and the possibility of a type II error does exist. As our sample size addresses our main outcome variable, it is our view that larger scale studies need to be undertaken to further investigate these relationships, which would furthermore include comparison between men and women, offering important information in regards to sex-related differences.

We considered the confounding effects of current smoking status and past smoking history between our two groups in deciding on selection criteria for the study. We ruled out the known immediate autonomic and cardiovascular effects of nicotine on vascular tone by requiring all COPD subjects to have quit smoking >1 year prior to entering the study. Because little is known about the cerebrovascular effects of smoking in COPD patients, we first wanted to identify outstanding differences between a COPD patient and a matched control. Indeed, a third ex-smoker control group would provide a valuable comparison.

CONCLUSION

This study is the first to show altered cerebrovascular responses to hypercapnia in women with moderate, smoking-related COPD. We show that increased oxidative stress in COPD patients, and we believe that this may contribute to the cerebrovascular impairments observed in these patients. Future research is needed to address interventional strategies aimed at minimizing systemic oxidative stress, thus providing direct evidence of the relationship between oxidative stress and cerebrovascular function.

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REFERENCES


Table 1. Subject characteristics

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<th>Controls (n=10)</th>
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<td><strong>Physical Characteristics</strong></td>
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<tr>
<td>Age, years</td>
<td>69.4 ± 4.3</td>
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<td>BMI, kg·m⁻²</td>
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<td>Total Cholesterol (mmol·L⁻¹)</td>
<td>5.16 ± 0.86</td>
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<td>HDL Cholesterol (mmol·L⁻¹)</td>
<td>1.66 ± 0.41</td>
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<td>Estradiol (pmol·L⁻¹)</td>
<td>69 ± 32</td>
<td>52 ± 16</td>
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<td>Progesterone (nmol·L⁻¹)</td>
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<td>Smoking Pack-years</td>
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<tr>
<th><strong>Lung Function</strong></th>
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<td>DL_co, ml·min⁻¹·mmHg⁻¹</td>
<td>12.1 ± 3.6 †</td>
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Abbreviations: BMI, Body mass index; HDL, High-density lipoprotein; FEV\textsubscript{1}, Forced vital capacity in 1 second; FVC, Forced vital capacity; TLC, total lung capacity; RV, Residual volume; FRC, Functional residual capacity; IC, Inspiratory capacity; DL\textsubscript{CO}, Diffusion lung capacity. Significantly different from Controls at P ≤ 0.05 (*) and P ≤ 0.01 (†).
Table 2. Comparison of end-tidal and capillary blood gases, hematocrit, and acid-base balance between COPD and control groups.

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</tbody>
</table>

Abbreviations: PcCO₂, Pressure of CO₂ from capillary blood; PcO₂, Pressure of O₂ from capillary blood; PETCO₂, Pressure of end-tidal CO₂; PETO₂, Pressure end-tidal O₂; HCO₃⁻, Bicarbonate ion; ctHb, concentration total hemoglobin.
Table 3. Physiological Variables at Rest and During Hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>Baseline Isocapnia</th>
<th></th>
<th>Hypercapnia</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COPD</td>
<td>Controls</td>
<td>COPD</td>
<td>Controls</td>
</tr>
<tr>
<td>n = 8</td>
<td>n = 10</td>
<td>n = 8</td>
<td>n = 10</td>
<td></td>
</tr>
</tbody>
</table>

**Respiratory**

- **PETCO₂**, mmHg: 34.0 ± 2.6, 35.2 ± 3.1, 41.6 ± 2.7, 43.0 ± 3.3 †
- **PETO₂**, mmHg: 90.1 ± 3.9, 88.8 ± 6.6, 90.3 ± 4.3, 89.0 ± 7.5
- **Vₑ**, L·min⁻¹: 7.6 ± 1.3, 8.2 ± 1.7, 14.5 ± 5.5, 23.3 ± 9.5 † ‡
- **Bᶠ**, breaths·min⁻¹: 14 ± 4, 12 ± 4, 16 ± 3, 16 ± 4 †
- **Vₑ/Tₑ**, L·breath⁻¹: 0.59 ± 0.18, 0.72 ± 0.22, 0.95 ± 0.38, 1.50 ± 0.61 † ‡
- **Vₑ/Tₑ/Tₑ**, L·sec⁻¹: 0.34 ± 0.11, 0.34 ± 0.07, 0.59 ± 0.21, 0.82 ± 0.33 † ‡
- **Tₑ/Tₑ/Tₑ**, L·sec⁻¹: 0.38 ± 0.05, 0.41 ± 0.05, 0.39 ± 0.05, 0.43 ± 0.04
- **Vₑ/MVV**, %: 18 ± 5 *, 10 ± 3, 32 ± 8, 26 ± 10 † ‡

**Cardiovascular**

- **MAP**, mmHg: 86.6 ± 9.8, 87.6 ± 12.3, 92.8 ± 9.2, 97.7 ± 12.2 †
- **HR**, beats min⁻¹: 67 ± 11, 67 ± 9, 70 ± 11, 71 ± 10
- **SaO₂**, %: 94 ± 2 *, 96 ± 1, 95 ± 2, 96 ± 1

**Cerebrovascular**

- **Vₑ**, cm·sec⁻¹: 46.9 ± 6.9, 46.6 ± 15.0, 55.8 ± 9.6, 63.3 ± 19.6 †
- **CVC**, cm·sec⁻¹·mmHg⁻¹: 0.55 ± 0.08, 0.54 ± 0.16, 0.60 ± 0.08, 0.65 ± 0.17 † ‡

**Abbreviations:** **PETCO₂**, Pressure of end-tidal CO₂; **PETO₂**, Pressure end-tidal O₂; **Vₑ**, Expired ventilation rate; **Bᶠ**, Breathing frequency; **Vₑ/Tₑ**, Volume of expired tidal breath; **Vₑ/Tₑ/Tₑ**, Volume of inspired tidal breath; **Tₑ**, Inspiratory time; **Tₑ/Tₑ/Tₑ**, Total time of respiratory duty cycle; **MVVₚredicted**, Predicted maximum voluntary ventilation; **MAP**, Mean arterial blood pressure; **HR**, Heart rate; **SaO₂**, Arterial oxygen saturation; **Vₑ**, Peak cerebral blood flow velocity; **CVC**, cerebrovascular
conductance (CVC = $\bar{V}p$/MAP). Significantly different from Controls at baseline; $P \leq 0.05$ (*). Significant main effect of hypercapnia at $P \leq 0.05$ (†). Significant group interaction with hypercapnia at $P \leq 0.05$ (‡)
Table 4. Plasma Oxidative Stress Markers, Antioxidant Enzyme Activity, and End-products of Nitric Oxide Metabolism in COPD and Controls

<table>
<thead>
<tr>
<th></th>
<th>COPD (n=8)</th>
<th>Controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidative Stress Markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-OHdG, µg·L⁻¹</td>
<td>9.6 ± 0.7 *</td>
<td>8.5 ± 1.3</td>
</tr>
<tr>
<td>MDA, µmol·L⁻¹</td>
<td>23.4 ± 4.7 †</td>
<td>13.1 ± 4.7</td>
</tr>
<tr>
<td>AOPP, µmol·L⁻¹</td>
<td>292.1 ± 125.1 †</td>
<td>134.8 ± 70.0</td>
</tr>
<tr>
<td>Nitrotyrosine, nmol·L⁻¹</td>
<td>93.1 ± 102.0</td>
<td>38.7 ± 34.2</td>
</tr>
<tr>
<td><strong>Antioxidant enzyme activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase, µmol·L⁻¹·min⁻¹</td>
<td>10.5 ± 6.6</td>
<td>9.0 ± 7.1</td>
</tr>
<tr>
<td>GPX, µmol·L⁻¹·min⁻¹</td>
<td>16.9 ± 4.9 †</td>
<td>9.5 ± 3.2</td>
</tr>
<tr>
<td><strong>NO End-products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOx, µmol·L⁻¹</td>
<td>4.9 ± 2.2 *</td>
<td>10.9 ± 6.4</td>
</tr>
</tbody>
</table>

**Abbreviations**: 8-OHdG, 8-Hydroxy-2’-deoxyguanosine; MDA, malondialdehyde; AOPP, advanced oxidation protein products; GPX, glutathione peroxidase; NOx, end-product of nitric oxide metabolism ([NO₃] + [NO₂]). Significantly different from Controls at p ≤ 0.05 (*). Significantly different from controls at P ≤ 0.01 (†).
Figure 1. Change in (A) peak cerebral blood flow velocity ($V_p$), (B) mean arterial pressure (MAP), and (C) ventilation ($V_e$) during incremental steps of euoxic hypercapnia in COPD patients and controls. COPD patients demonstrate a decreased cerebral blood flow response to hypercapnia ($P < 0.05$). Error bars represent SD.
Figure 2. Physiological responses to CO$_2$ in COPD patients and controls. Cerebrovascular and ventilatory sensitivity indices were calculated as the slope of the line relating peak cerebral blood flow velocity ($\bar{V}_P$) or ventilation ($\bar{V}_E$), respectively, to the increases in end-tidal CO$_2$ (+9 mmHg above rest). Means are presented with 95% confidence intervals.

A) Cerebrovascular sensitivity to CO$_2$.
Individual (small closed circles) and unadjusted mean (large closed circles) responses are plotted for both COPD and controls. The cerebrovascular sensitivity is decreased in the COPD group when comparing the unadjusted means (*P ≤ 0.01) (COPD: $y = 1.22x + 5.11$; Controls: $y = 2.16x − 31.03$). Once means are adjusted for oxidative stress (8-OHdG, MDA, and AOPP) (large open circles), no significant difference (NS) between groups exists.

B) Ventilatory sensitivity to CO$_2$.
Individual (small closed circles) and mean (large closed circles) responses are plotted for both COPD and controls. There is a trend to decreased ventilatory response during hypercapnia in COPD patients compared to controls. (COPD: $y = 0.89x + 6.28$; Controls: $y = 1.75 + 5.50$).